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In Vitro Replication, Packaging, and Transcription of the Segmented Double-Stranded RNA Genome of Bacteriophage φ6: Studies with Procapsids Assembled from Plasmid-Encoded Proteins

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The genome of the lipid-containing bacteriophage φ6 contains three segments of double-stranded RNA (dsRNA). We prepared cDNA copies of the viral genome and cloned this material in plasmids that replicate in *Escherichia coli* and *Pseudomonas phaseolicola*, the natural host of φ6. These plasmids direct the formation of viral proteins and the assembly of structures similar to viral procapsids containing proteins P1, P2, P4, and P7. We found that these particles are capable of taking up viral single-stranded RNA and synthesizing the minus strands to produce dsRNA structures. Once the dsRNA is formed, it is then used as a template for the production of viral plus strands in a reaction that resembles normal transcription. The particles were also capable of directly transcribing exogenous dsRNA. The replicase reactions were specific for φ6 RNA, were specific for procapsids, and resulted in substantial incorporation of product dsRNA into particles. These results offer strong support to a model in which genomic packaging is done by preformed procapsids.

Bacteriophate φ6 infects the plant pathogen *Pseudomonas phaseolicola* HB10Y (27). Three separate pieces of double-stranded RNA (dsRNA) compose its genome, which is located inside a polyhedral nucleocapsid (23). Virus particles contain one each of the three genomic segments, and the efficiency of plating of the virus is close to 1 (3). The mechanisms by which viruses containing segmented dsRNA genomes are able to package the various segments and to replicate them are not known. The nucleocapsid has RNA polymerase activity (20) and is covered by a lipid-containing membrane (27). It has been proposed that the assembly pathway involves the formation of a polyhedral procapsid which is then filled with one copy each of the three pieces of dsRNA per virion (3, 15). These filled procapsids would then be covered with a shell of protein P8 to become nucleocapsids which would be subsequently enveloped within the lipid-containing membrane (13).

The study of the phage assembly process has been facilitated by the cloning of cDNA copies of each genomic segment (16, 17). Plasmids containing the cDNA copy of genomic segment L are capable of directing the synthesis of proteins P1, P4, P7, and P2. These proteins are the constituents of the normal φ6 procapsid (15). The proteins assemble in both *Escherichia coli* and *P. phaseolicola* to form cagelike structures that resemble the viral procapsids in morphology, composition, and sedimentation behavior (10).

Here, we report that these particles have both replicase and packaging activities when incubated with φ6 message-sense RNA. The particles also exhibit transcriptase activities. These activities have the characteristics of those expressed by viral procapsids. This system should provide the basis for elucidating the mechanism of packaging of segmented genomes of dsRNA viruses.

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MATERIALS AND METHODS

Bacterial strains, phage, and plasmids. *E. coli* JM109 (Table 1) was used for procapsid production and maintenance of plasmids. A mutant of *P. phaseolicola* HB10Y (27) designated LM128 was also used.

The construction of several of the plasmids has been described in our original report of particle formation (10). The plasmids are described in Table 1, and the cDNA copies are illustrated in Fig. 1. Plasmid pLM570 was constructed by first inserting the *lac* gene of plasmid pMC1871 into pLM254, then inserting gene 1 in front of it. A segment of pLM362 containing genes 7 and 2 and gene 4 truncated at the *Hin*dIII site was then inserted between genes 1 and *lac* so that gene 4 was in frame with *lac*. A small amount of the fusion protein may be associated with isolated procapsid particles from this construction (see Fig. 8, lane b).

Some of the plasmids used a new set of vectors that we developed. The basic vector is pLM416, which is the P-type plasmid pRK290 with the multiple cloning sites of plasmid pLM254. This multiple cloning site region is derived from pUC8 but has a mutation in the promoter that allows higher expression in pseudomonads (10). This plasmid was further modified by inserting the 1.7-kilobase-pair *Eco*RI fragment of pMC9 into its *Bgl*II site. The pMC9 fragment contains a gene for high-level expression of *lac* repressor. The resulting vector is named pLM523. It was used for the construction of plasmid pLM532, which has a cDNA copy of the entire L segment inserted into the *Pst*I site. This plasmid can be propagated in *P. phaseolicola* and results in the production of procapsids. It is more stable in *Pseudomonas* species than is pLM450, which has the same L segment cloned into plasmid pLM416. Plasmid pLM450 is difficult to maintain in *P. phaseolicola* even in the presence of another compatible plasmid carrying *lacI*^{q}.

Preparation of procapsids. Either *E. coli* JM109 or *P. phaseolicola* LM128 carrying the appropriate cDNA plasmid

TABLE 1. Bacterial strains and plasmids

Bacteria or plasmid	Remarks	Reference or source
Bacteria		
P. phaseolicola LM128	Host of φ6, derivative of HB10Y	27
E. coli JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* λ⁻ Δ(*lac-proAB*)(F′ *traD36 proAB lacI*^{q}ZΔM15)	J. Messing
Plasmids		
pUC8	*bla*, *lacZ′* region with multiple cloning sites	28
pMC9	*lacI*^{q} in *Eco*RI site of pBR322	12
pLMF306	Fragment 306 in pBR322 *Pst*I site; orientation same as *bla*	10
pLMF308	Fragment 308 in pBR322 *Pst*I site; orientation same as *bla*	10
pLMF309	Fragment 309 in pBR322 *Pst*I site; orientation opposite to *bla*	10
pLM254	*bla str lacZ′*, chimera of pUC8 and pKT230	16
pRK290	Broad-host-range vector, *tet*	4
pLM358	Fragment 309 in *Pst*I site of pLM254 produces P1 and P4	This study
pLM362	Complete L segment in *Pst*I site of pUC8 (reverse orientation)	10
pLM369	Complete L segment in *Pst*I site of pUC8 produces P1, P4, P7, and P2	10
pLM375	Complete L segment in *Pst*I site of pLM254 produces P1, P4, P7, and P2	10
pLM416	MCS of pLM254 in pRK290	This study
pLM450	L segment in *Pst*I site of pLM416 produces P1, P4, P7, and P2	This study
pLM523	*lacI*^{q} segment of pMC9 in *Bgl*II site of pLM416	This study
pLM532	L segment in *Pst*I site of pLM523 produces P1, P4, P7, and P2	This study
pLM570	L segment with P4:*lac* fusion in pLM254 produces P1, P7, P2, and P4:*lac* fusion	This study
pLM574	pLM375 with a deletion from *Eco*RI to *Nru*I produces P1, P4, and P2	This study
pLM609	pLM375 with a deletion from *Xba*I to *Sph*I produces P1, P4, and P7	This study

was incubated overnight in LB medium at 26°C. The culture was diluted in the morning to 2×10^8 cells per ml, allowed to double in density, and then incubated with 1 mM isopropyl-β-D-thiogalactopyranoside for 4 h. The cells were harvested and lysed in a French pressure cell at 6,000 lb/in^2. The lysates were centrifuged at 8,000 × *g* for 5 min, and the supernatant liquid was applied to gradients containing 10 to 30% sucrose, 10 mM potassium phosphate (pH 7.3), and 1 mM magnesium chloride. The tubes were spun for 90 min at 23,000 rpm in a Beckman SW41 rotor. The contents of the tubes were fractionated, and samples were analyzed in sodium dodecyl sulfate (SDS)-15.5% polyacrylamide gels as described previously (14). Peak fractions were combined and preserved at −70°C. Frozen procapsids maintained activity for at least 6 months. Figure 8 shows the composition of the peak fractions of the procapsid preparations used in this study. The procapsids derived from *P. phaseolicola* were more highly contaminated with host proteins than were the samples from *E. coli* because the amount of viral protein synthesis was greater in the latter cultures.

Sources of RNA. Nucleocapsids were prepared from purified preparations of φ6 and incubated under the conditions specified by Emori et al. (5). The resulting RNA was a mixture of single-stranded RNA (ssRNA) and dsRNA. This was resolved on a cellulose column with elution buffers containing decreasing concentrations of ethanol (8). ssRNA was eluted at 20% ethanol (see Fig. 3). dsRNA was extracted from intact virions with phenol. Bacteriophage MS2 RNA and *E. coli* rRNA were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). dsRNA M1 and L1A from killer yeasts, and dsRNA from rotavirus were generous gifts from T. Fujimura and R. Wickner.

RNA polymerase reaction conditions. Standard reaction mixtures contained 25 μg with 50 mM Tris (pH 8.2), 4 mM magnesium chloride or 3 mM manganous chloride, 100 mM ammonium acetate, 20 mM NaCl, 5 mM KCl, 5 mM dithiothreitol, 0.1 mM EDTA, 1 mM each of ATP, GTP, and CTP, 0.1 mM UTP, 5% polyethylene glycol 4000, and 1 mg of macaloid (7). α-^{32}P-labeled UTP (20 μCi) was also included. There was usually about 2 μg of procapsid and 1 μg of RNA per reaction. The reaction was run at 27°C for 90 min. It was stopped by the addition of 7.5 μl of 20 mM EDTA–0.3% SDS and then extracted with phenol. RNA was analyzed on composite agarose-acrylamide gels (24). In some experiments (see Fig. 7), both magnesium and manganous ions were present in the reaction mixture.

Packaging assay. Frozen procapsids produced in *E. coli* JM109 carrying plasmid pLM450 were thawed and incubated in a replicase reaction with 2 mM magnesium chloride and 3 mM manganous chloride. Immediately after the reaction, the material was applied to a sucrose gradient that contained 0.3 M ammonium chloride in addition to the usual components and centrifuged at 23,000 rpm for 45 min and fractionated. Aliquots were analyzed on composite gels for RNA synthesis and on SDS-15.5% polyacrylamide gels for the presence of protein P1 as an indication of the position of procapsids.

Gel electrophoresis. Samples were analyzed on SDS-15.5% polyacrylamide gels for protein content (14). These gels were stained with silver or Coomassie blue. Samples containing RNA were analyzed on composite gels of 2% acrylamide–0.5% agarose following the description of Studier (24). It was necessary to add carrier dsRNA to samples that were used in replicase assays because small amounts of dsRNA run anomalously on these gels. These gels were stained with ethidium bromide or subjected to autoradiography. Strand-separating gels were run by the procedure of Pagratis and Revel (18a). These gels were stained with ethidium bromide, dried, and autoradiographed.

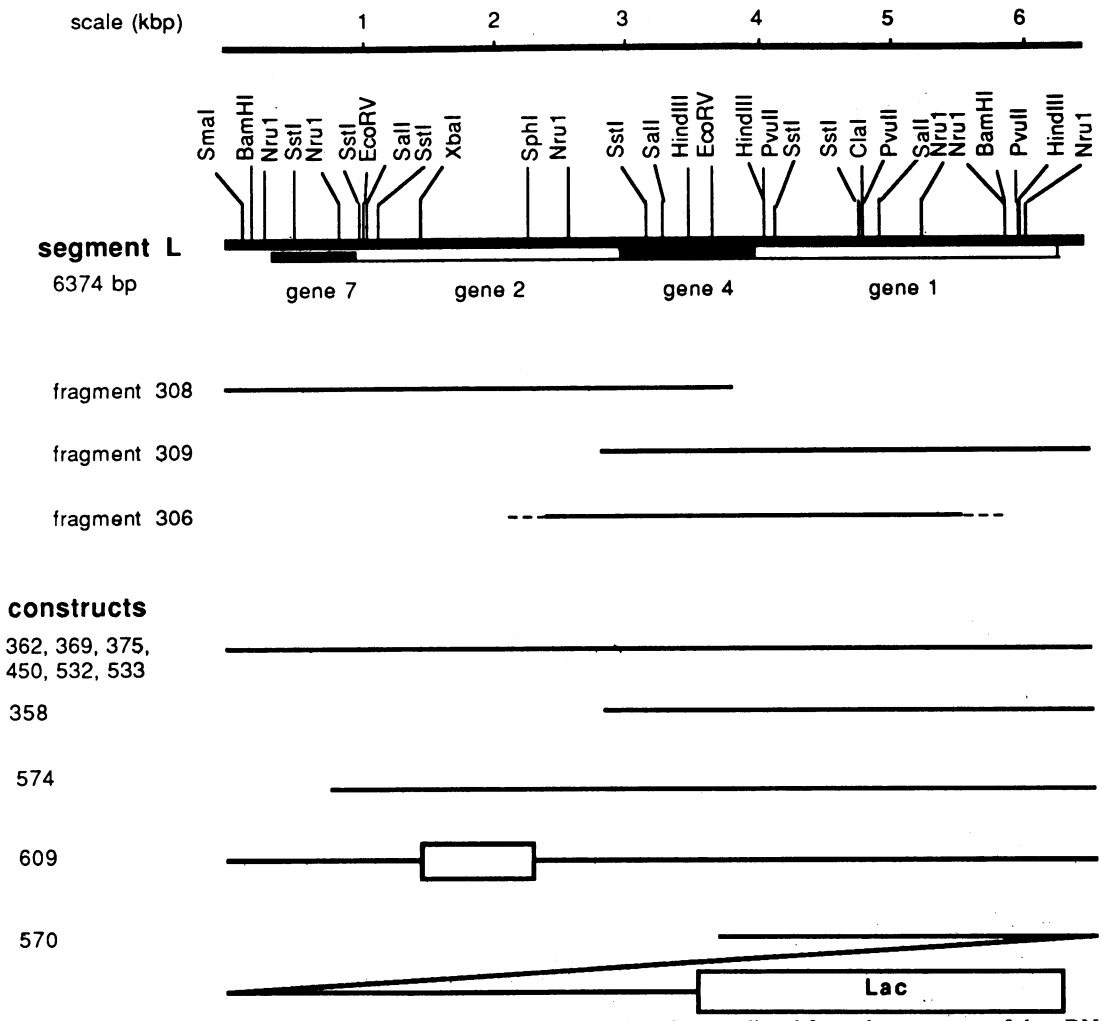

FIG. 1. Map of genomic segment L and the cDNA fragments. The restriction sites predicted from the sequence of the cDNA copies of genomic segment L are presented along with the compositions of the inserts used in this study. Fragments 308, 309, and 306 are original cDNA isolates (17). Construct 358 is derived from fragment 309. The full copies such as 362, 369, 450, and 532 are derived from the same construct derived from fragments 308, 309, and 306 as described previously (10). They have intact reading frames, and the genes are capable of complementation. Construct 574 is a deletion between the *Eco*RI site of the vector pLM375 and the *Nru*I site at position 836 in gene 7. Construct 609 contains a deletion from *Xba*I to *Sph*I and does not produce P2. Construct 570 has a fusion of the *lac* gene with the N-terminal portion of gene 4. Gene 1 is transposed to a position upstream of gene 7. kbp, Kilobase pairs.

RESULTS

Optimization of polymerase activities. Particles were formed in *E. coli* carrying plasmid pLM450 (Table 1; Fig. 1 and 2), which specifies the products of genes 1, 4, 7, and 2. The cells were lysed in a French press, and the lysate was fractionated on a sucrose gradient. The fractions containing proteins P1, P2, P4, and P7 were combined and incubated with ϕ6 ssRNA (Fig. 3).

We found that incubation of particles with ribonucleotide triphosphates, ssRNA, magnesium and/or manganese ions, ammonium acetate, and polyethylene glycol was sufficient to produce labeled dsRNA when α-^{32}P-labeled UTP or CTP was included in the reaction mix. The reaction depended on particle concentration and had an optimum at a magnesium concentration of 3 mM or a manganese concentration of 2 mM (Fig. 4). The reaction depended on ammonium acetate, and the optimum in our reaction was 100 mM. The optimum pH was about 8.7, and the concentration of polyethylene glycol 4000 was 5%. We used pH 8.2 for most of our reactions. The reaction depended on the presence of all four ribonucleotide triphosphates (data not shown). The reaction was not saturating for either procapsid concentration or RNA concentration.

Products of polymerase reactions. The product of the reaction with a ssRNA template was dsRNA having the same migration properties as normal viral dsRNA. When subjected to denaturation and electrophoresis on a strand-separating gel, the product contained labeled minus strands (Fig. 5). This is consistent with the expectation that replication involves the formation of a negative-sense strand on the positive-sense template (6). Reactions in the presence of magnesium resulted in the production of dsRNA; however, reactions in the presence of manganese resulted in the appearance of ssRNA as well (Fig. 4), indicating that the dsRNA that is formed is also competent to support the transcriptase reaction. The appearance of labeled

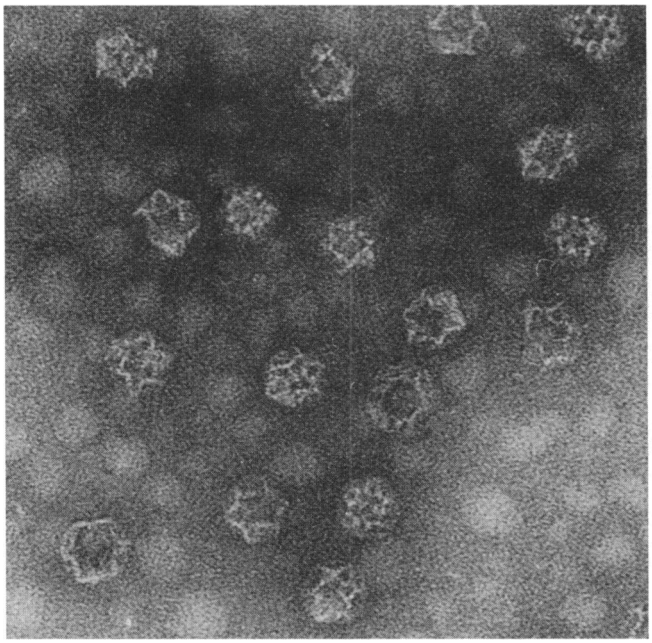

FIG. 2. Electron micrograph of procapsids. Procapsids were isolated from *E. coli* JM109 containing pLM450 as described in Materials and Methods. The particles were isolated from a sucrose gradient and freed of sucrose by using a spin column of Sephadex G-50. The sample was then applied to a carbon-coated grid and stained with ammonium molybdate. Magnification, ×181,000.

ssRNA indicates that the transcriptase reaction can proceed for at least two rounds, since it has been shown that transcription in φ6 is by a strand displacement mechanism (25, 27).

When the reaction was primed with dsRNA isolated from virions, the labeled products were dsRNA. This was expected since the transcriptase reaction appears to involve displacement of the parental plus strand by the nascent one (25, 26). A single round of transcription will produce only unlabeled ssRNA. This reaction was much more dependent on manganese than was the reaction primed by ssRNA. Very little transcription was seen when magnesium was the only divalent cation supplied. The conditions for the reaction were otherwise similar to that primed by ssRNA. The labeled products of the reaction primed by dsRNA were analyzed in strand-separating gels and were found to be labeled in the plus strands, consistent with a transcription reaction (Fig. 5).

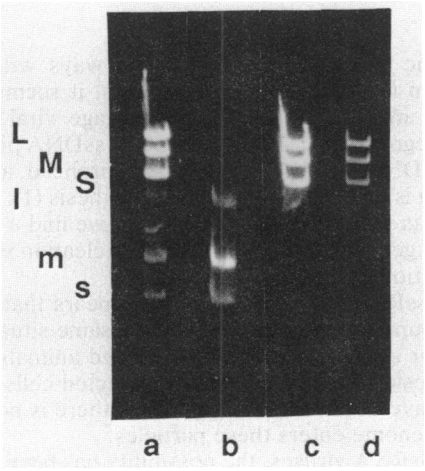

FIG. 3. Purification of ssRNA from a transcription reaction. A composite SDS-agarose-polyacrylamide gel was stained with ethidium bromide to show the separation of ssRNA and dsRNA by cellulose columns. Lanes: a, unfractionated RNA from the transcription reaction; b, pooled material eluting from the column at 20% ethanol; c and d, material eluting at 0% ethanol. L, M, and S refer to the dsRNA segments, and l, m, and s refer to their ssRNA transcripts.

The reactions were moderately specific with respect to the source of template RNA (Fig. 6). No labeled product was formed when the template was bacteriophage MS2 RNA. However, *E. coli* RNA resulted in the formation of labeled product that migrated as the dsRNA form of 16S rRNA. Upon denaturation, the product ran with 16S RNA. There was no apparent reaction with 23S RNA. This replication of 16S RNA was seen in the presence of manganese ions. In a reaction with magnesium ions, 16S RNA did not act as a template. The transcriptase reaction also shows variable specificity. Both L1A dsRNA and M1 dsRNA of the yeast killer system (9) are poor templates (data not shown). RNA extracted from rotavirus did not serve as an effective template.

The activities copurified with the peak of procapsid protein in sucrose gradients. When a lysate of *E. coli* containing procapsids was fractionated on a sucrose gradient and the fractions were tested for replicase, transcriptase, and protein content, we found that the major activity coincided with the major peak of procapsid proteins (data not shown).

The replicase product is packaged. A reaction mixture that used φ6 ssRNA as the template and complete procapsids isolated from *E. coli* was fractionated on a sucrose gradient. The fractions were analyzed for protein and for radioactivity (Fig. 7A). The procapsids were found to sediment as expected. The labeled dsRNA was found in a peak sedimenting ahead of the empty procapsids. The major fraction of the peak (fraction 14) contained labeled segments S and M. The material in fraction 15 contained more L relative to S and M than did fraction 14. Some material remained at the top of the gradient, and in other experiments we found labeled ssRNA at the top of the gradient. In many of our experiments, most of the labeled dsRNA was particle associated.

When a reaction mixture that used φ6 dsRNA as the template was treated in the same way, the procapsids sedimented as before, but the labeled dsRNA was found at the top of the gradient (Fig. 7B). When the template was 16S rRNA from *E. coli*, the product, labeled dsRNA, was found at the top of the gradient and not associated with particles.

Reactions of incomplete procapsid particles. We prepared plasmids containing less than the complete cDNA copy of genomic segment L (Fig. 1; Table 1). With this collection, it was possible to produce particles that contained proteins P1 and P4; P1, P4, and P7; or P1, P4, and P2 (Fig. 8). Particles that do not contain P4 are unstable and difficult to isolate (10). However, we isolated particles using a construction in which the N terminus of protein P4 is fused to β-galactosidase. These are more stable than the particles that are missing P4 completely. When the replicase reaction was performed with the incomplete particles (Fig. 9), the particle missing P2 and P7 did not have activity, nor did the particle that is missing P2. The particle that is missing P7 had

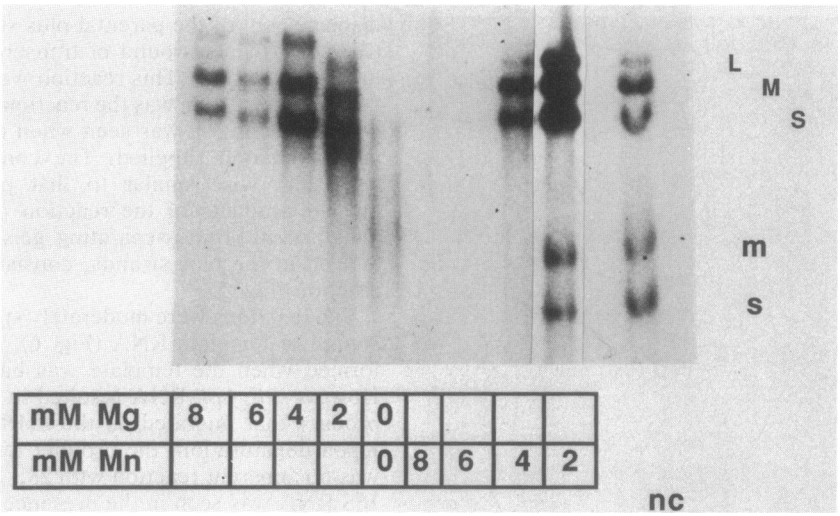

FIG. 4. In vitro replication of ϕ6 ssRNA. Replicase reactions were performed with complete procapsids encoded by pLM450 in *E. coli* JM109. Standard reaction conditions were used with purified ssRNA (lane b of Fig. 3), but the divalent cation concentrations were varied as indicated below the autoradiogram. Lane nc contains labeled RNA synthesized in vitro with purified viral nucleocapsids. L, M, S, m, and s are as described in the legend to Fig. 3.

replicase activity, but at a lower level than the complete particle. The particle that is deficient in P4 due to gene fusion also had replicase activity at a level approximately 5% that of complete particles. It was also found that complete particles isolated from *P. phaseolicola* had the same level of activity as those isolated from *E. coli*. Material isolated from gradients containing lysates of either *E. coli* or *P. phaseolicola* that do not carry plasmids showed no reaction. The particles that were missing P7 were able to effectively package the dsRNA formed from ssRNA templates. The particles that were missing P4 could not be assayed for packaging because the level of synthesis was too low. Complete particles were able to transcribe exogenously added dsRNA (Fig. 6); however, this activity was less than 10% that of replicase activity with the same amount of RNA. Particles that are missing P7 or have a fusion of P4 with β-galactosidase show normal transcriptase activity with exogenous dsRNA (data not shown). Particles missing P2 did not show transcriptase activity.

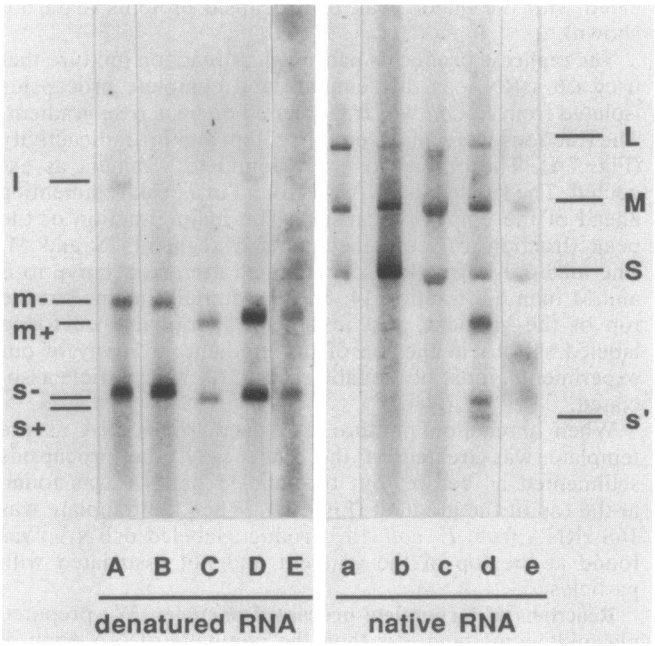

FIG. 5. Demonstration of positive- and negative-strand synthesis. Reaction products such as those illustrated in Fig. 4 were subjected to electrophoresis on a strand-separating gel either with or without denaturation before loading. Shown is the autoradiogram of a gel with the products of a reaction with ssRNA template and 2 mM manganous chloride (lanes A, a, B, and b) and with dsRNA template and 2 mM manganous chloride (lanes C, c, E, and e). Lane d contains the product of an in vitro transcription reaction with viral nucleocapsids. L, M, and S refer to the bands for dsRNA. l, m, and s refer to the ssRNA bands. s′ is an isoconformer of s, described previously by Pagratis and Revel (18a). Lanes E and e were exposed for three times as long as the other lanes.

DISCUSSION

Genomic packaging occurs in two ways with spherical viruses. In the dsDNA bacteriophages, it seems clear that proheads are able to select and package viral DNA with various degrees of fidelity (2). Even the ssDNA phage ϕX174 packages DNA into an already formed prohead, although the packaging is concurrent with DNA synthesis (1). Only in the filamentous ssDNA bacteriophages do we find a situation in which the genomic DNA is used as a nucleation structure for the formation of the viral capsid.

In the ssRNA bacteriophages, it appears that the capsid forms around the genomic RNA. The same situation seems to hold for ssRNA viruses of plants and animals. Although empty capsids have been found in infected cells and empty capsids have been assembled in vitro, there is no indication that the genome enters these particles.

In the dsRNA viruses, the possibility has been considered that genomic packaging could utilize either entry into preformed procapsids or procapsid condensation around the genomic dsRNA or its ssRNA precursors. An additional complication in the mechanism for most dsRNA viruses is the matter of their complex genomes. Reovirus has 10 genomic segments, and rotavirus has 11 segments. Bacteriophage ϕ6 has three segments, and we have shown that the

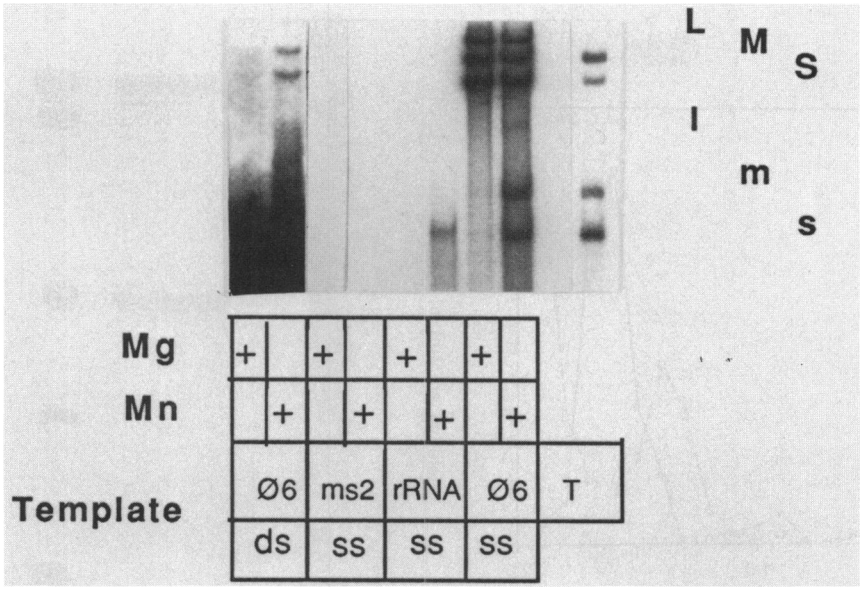

FIG. 6. Synthesis of RNA by procapsids with various templates. Autoradiogram of a composite gel showing radioactive products formed in the reaction of complete procapsids with dsRNA or ssRNA. Reactions were with φ6 dsRNA, with MS2 RNA, with *E. coli* RNA, and with φ6 ssRNA. The reactions were run under standard conditions with either 3 mM manganous chloride or 2 mM magnesium chloride. The dsRNA reaction lanes were exposed for 30 times as long as the ssRNA reaction lanes. φ6 dsRNA segments S, M, and L are 2.9, 4.1, and 6.4 kbp respectively (13). 16S rRNA is 1.5 kbp. Lane T contains a sample of φ6 RNA isolated from a transcriptase reaction of purified viral nucleocapsids.

efficiency of packaging in vivo is close to perfect (3). If the procapsid structure condensed around the genomic segments or their precursors, it would be necessary to explain how the proper three segments would be collected. Similarly, if the genomic segments were packaged in a preformed procapsid, it would be necessary to determine whether they enter in a particular order and how the system is able to chose one of each instead of multiples.

Whereas empty procapsids can be isolated from cells infected with the proper mutants of dsDNA bacteriophages because procapsid formation is a late function, φ6 procapsids are synthesized early in infection and genomic replication depends on procapsids. Therefore, mutations in the genes for procapsid proteins result in poor RNA synthesis and therefore poor production of procapsids. Since there is no distinguishing characteristic between virgin procapsids and those that have lost their genomic content, it would be difficult to know whether empty procapsids prepared from infected cells had previously participated in packaging. For killer particles in yeasts, it was shown that particles could lose their genomic content and still be capable of replicase or transcriptase activity (9); however, these particles have not been shown to package either the ssRNA or the dsRNA. A system has also been developed for in vitro replication of rotavirus RNA (21, 22). This system was also shown to replicate exogenous ssRNA; however, it has not yet been shown that the product is packaged. Our strategy was to use recombinant particles because these could not have been exposed to viral RNA during their development. They would have been exposed to the mRNA encoded by the plasmid and bearing the entire sequence of genomic segment L, but these molecules would probably have very different ends than the normal viral RNA. We had previously shown that particles formed from cDNA-encoded genes were similar in composition and morphology to particles isolated from infected cells (10).

The replicase reaction that we report here appears to have the characteristics expected for the normal φ6 mechanism. Plus strands of ssRNA are taken up by the particles, and minus strands are synthesized. We do not yet know whether the synthesis takes place concurrently with uptake or afterward. The new RNA is in the particles and ultimately appears in the ratios found in mature virions. The dsRNA that is packaged in this way is capable of being transcribed to form labeled plus strands. In contrast, exogenous dsRNA is transcribed, but the labeled dsRNA is not found in particles. We have found that filled procapsids prepared as described in this report can infect spheroplasts of *P. phaseolicola* if first incubated with protein P8 (V. Olkkonen and P. Gottlieb, unpublished data). This result supports the contention that the normal route of assembly involves the uptake of ssRNA by the empty procapsid concurrent with or followed by synthesis of the minus strand to form dsRNA and finally by transcription to form plus strands of ssRNA. It seems reasonable that other dsRNA viruses would use the same general mechanism.

The finding that procapsids prepared from *E. coli* are as effective as those from *P. phaseolicola* in both the replicase and packaging reactions suggests that specific host factors are not needed for these reactions. However, the particles isolated from sucrose gradients are contaminated with host proteins to a degree, and it is possible that some of these are contributing to the reactions. It seems clear that there are no requirements for host proteins in abundance.

We still do not know the roles of the particular procapsid proteins in the replicase and transcriptase reactions. Protein P1 is the basic structural component of the procapsid. Without it there is no structure. Protein P2 appears to be essential for RNA synthesis, and it is tempting to think that it contains the active site of the polymerase. It is found in about 20 copies per virion compared with 120

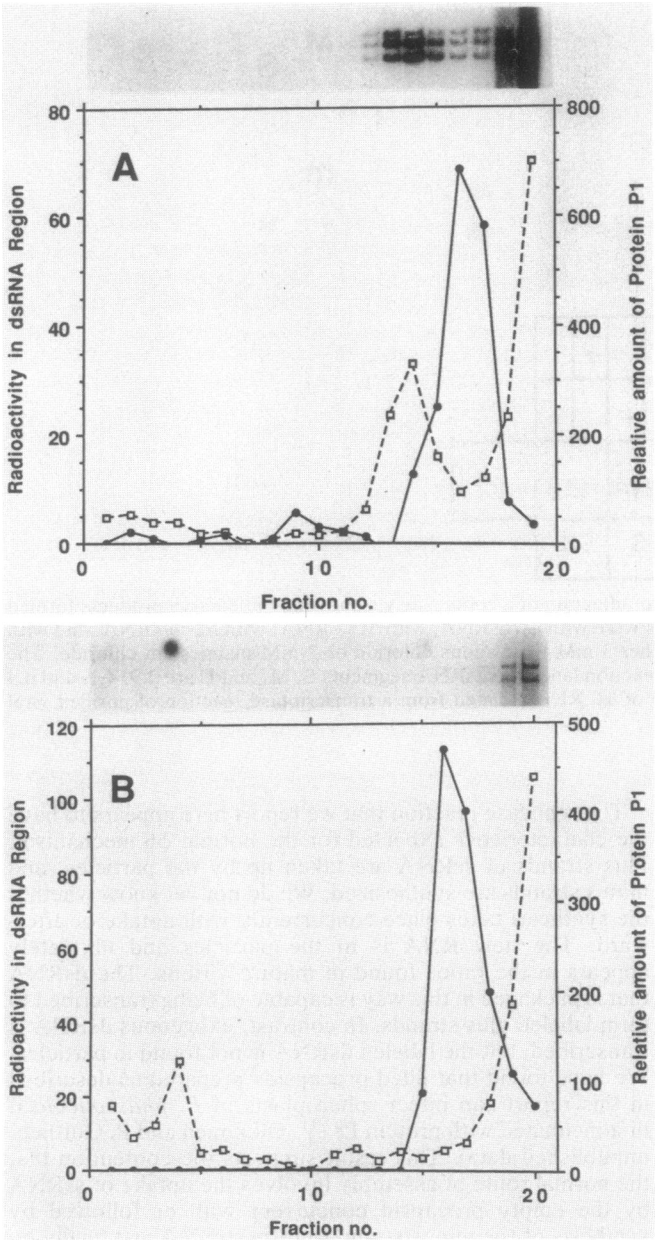

FIG. 7. Association of labeled dsRNA with particles. (A) Procapsids were incubated with ssRNA, and the products were centrifuged in a sucrose gradient containing 0.3 M ammonium chloride. The radioactivity in dsRNA was measured for each fraction (□). Fractions were analyzed on an SDS-15.5% polyacrylamide gel and stained with silver, and the amount of protein P1 in each fraction was determined by densitometry (●). The autoradiogram showing the labeled dsRNA in the fractions is above the graph. (B) Procapsids were incubated with dsRNA, and the products were centrifuged in a sucrose gradient containing 0.3 M ammonium chloride. Symbols are as in panel A.

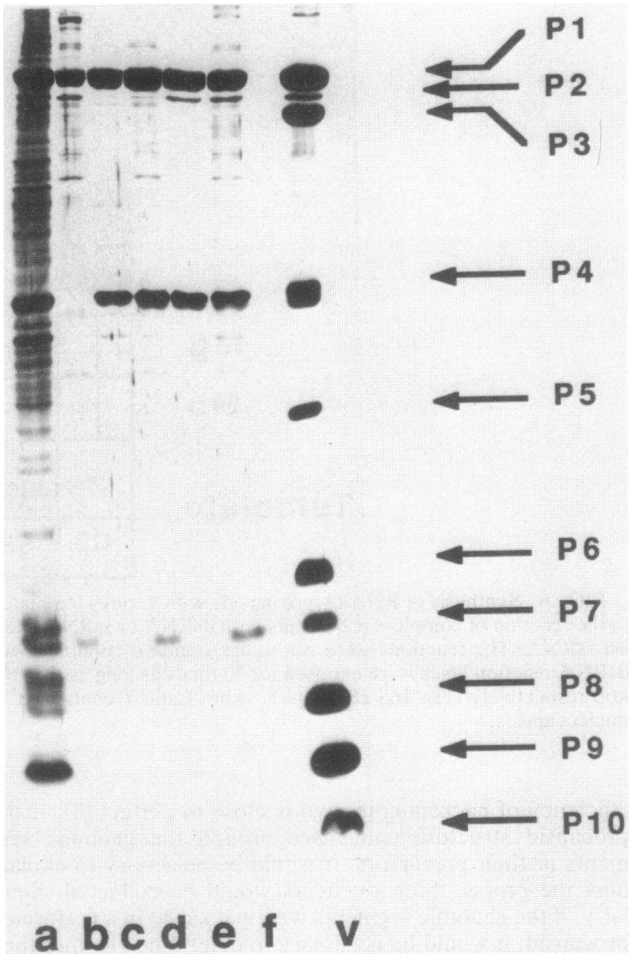

FIG. 8. Composition of plasmid-encoded procapsids. Shown is an SDS-15.5% polyacrylamide gel stained with silver showing the procapsid fractions isolated from sucrose gradients of a lysate of *P. phaseolicola* carrying pLM532 (P1, P2, P4, P7) (lane a) and lsyates of *E. coli* carrying plasmids pLM570 (P1, P2, P7, P4:*lac* fusion) (lane b), pLM358 (P1, P4) (lane c), pLM609 (P1, P4, P7) (lane d), pLM574 (P1, P2, P4) (lane e), and pLM450 (P1, P2, P4, P7) (lane f). Lane v contains proteins from purified virions.

for P1 and P4 (3), and it has sequence similarity to other viral RNA polymerases (11). The finding that P7 is somewhat dispensable indicates that it plays a facilitating role rather than an essential one. However, we have genetic evidence that P7 must function in the normal infection process. Nonsense mutations in gene 7 are polar on the expression of P2. These mutants are complemented by plasmids containing intact genes 7 and 2 but not by plasmids containing only gene 2 (unpublished observation). The observation that transcription of exogenous dsRNA is not as diminished as replication in particles missing P7 suggests that this protein has an enhancing role in replication or packaging.

It should be possible with the system described in this report to manipulate the composition of the procapsid proteins to determine their participatory roles in viral development. We know that protein P4 has nucleoside triphosphate phosphohydrolase activity (unpublished observations). The role of this activity in replication or packaging can now be addressed. The low replicase activity seen with the particles that are missing normal P4 indicates that P4 is important, but it is not yet clear whether its role is in packaging, replication, or both. As for particles missing P7, the effect of P4 seems to be greatest on replication.

We found that the ϕ6 replicase has high activity with *E. coli* 16S rRNA as the template in the presence of manganese. The reaction with magnesium was more stringent. In neither case was MS2 RNA copied. Thus, the ϕ6

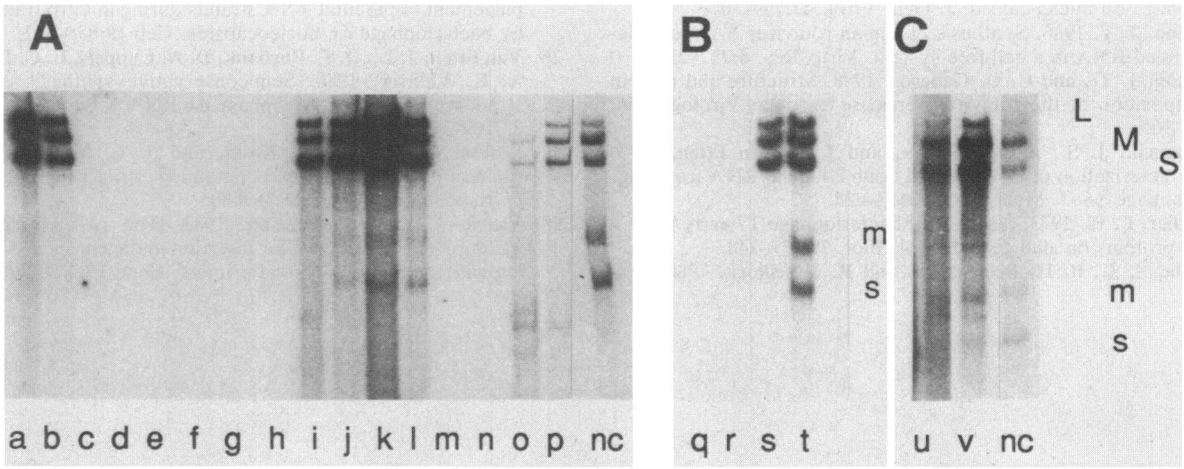

FIG. 9. Activities of incomplete particles. The particles shown in Fig. 8 and some others were tested for ability to synthesize RNA with a φ6 ssRNA template. A composite gel is shown with the reaction products from complete particles (P1, P4, P7, and P2) from *P. phaseolicola* (lanes a and b), gradient fractions of a pseudomonas lysate not containing procapsids (lanes c and d), particles containing P1 and P4 from *P. phaseolicola* (lanes e and f), particles containing P1 and P4 from *E. coli* JM109 (lanes g and h), complete particles from *P. phaseolicola* (lanes i and j), complete particles from *E. coli* JM109 (lanes k and l), gradient fractions of a JM109 lysate not containing procapsids (lanes m and n), and particles containing P1, P4, and P2 (lanes o and p). Lanes nc contain labeled transcripts derived from viral nucleocapsids. Also shown are particles from *E. coli* JM109 containing P1, P4, and P7 (lanes q and r), complete particles from JM109 in a replicase reaction with magnesium instead of manganese ions (lane s), complete particles in a standard reaction (lane t), particles containing P1, P2, P7, and the fusion of P4 and *lac* (lane u), and complete particles (lane v). Panels A, B, and C are from different reaction sets and different gels.

replicase seems somewhat more discriminating than that of Qβ, for which it has been found that many heterologous species of RNA could be copied in the presence of manganese ions (18, 19).

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